

T Cell Receptor β Chain Gene Usage in Endemic Pemphigus Foliaceus (Fogo Selvagem)

Achim K. Moesta, Mong-Shang Lin,* Luis A. Diaz,† and Animesh A. Sinha

Department of Dermatology, Weill Medical College of Cornell University, U.S.A.; *Department of Dermatology, Medical College of Wisconsin, U.S.A.; †Department of Dermatology, University of North Carolina, U.S.A.

The trimolecular complex comprised of the major histocompatibility complex, peptide antigen, and the T cell receptor is a requisite for T cell activation in normal and autoimmune responses. T cell receptor analysis is critical to further our understanding regarding mechanisms of T cell epitope selection and autoimmune initiation and progression and may help to identify targets for immunotherapy. Pemphigus foliaceus is an autoimmune blistering skin disease characterized by intraepidermal blisters and circulating autoantibodies directed against desmoglein 1, a 160 kDa transmembrane desmosomal molecule expressed in keratinocytes. As tissue damage is mediated by anti-desmoglein 1 antibodies, an initial T cell response is a likely requirement for autoantibody generation in this disease. To elucidate the role of pathogenic T cells in autoimmunity further, we have directly characterized the T cell receptor of T cells derived from pemphigus foliaceus patients. Complementary DNA was isolated from 17 desmoglein 1 specific T cell clones generated from

pemphigus foliaceus patients by clonal expansion *in vitro*. To analyze the T cell repertoire, a panel of primers, collectively specific for the known human T cell receptor β variable region (TCRBV) families were paired with a constant region primer to polymerase chain reaction to amplify one distinct T cell receptor β variable region allele for each T cell clone studied. Polymerase chain reaction products were sequenced to determine exact β chain gene usage. In the 17 clones tested, 10 distinct T cell receptor β variable region usages and nine T cell receptor β joining gene segment usages were identified. Furthermore, T cell receptor β variable region and β joining usage did not appear to be random, but oligoclonal in nature, with some preference shown for T cell receptor β variable region 5S1 and T cell receptor BJ2S5. **Key words:** polymerase chain reaction/clone cells/receptor/antigen/T-cell/gene rearrangement/beta-chain T cell antigen receptor/DNA/complementary. *J Invest Dermatol* 119:377–383, 2002

Pemphigus foliaceus (PF) is an autoimmune blistering skin disease that can be broadly subclassified into two types. Nonendemic PF is found throughout the world, whereas endemic forms have been found locally in Brazil, Columbia, and Tunisia (Robledo *et al*, 1988; Diaz *et al*, 1989; Morini *et al*, 1993; Bastuji-Garin *et al*, 1995). Endemic and nonendemic forms are clinically, histologically, and immunologically indistinguishable. Endemic forms are usually diagnosed based on epidemiology, high frequency of occurrence among children and young adults, and family history (Squiquera *et al*, 1988).

Fogo selvagem (FS) is the endemic form of PF found in certain regions of Brazil. Susceptibility to FS is associated with human leukocyte antigen (HLA) alleles DRB1*0102, DRB1*0404, DRB1*1402, and DRB1*1406, with a relative risk as high as 14 for carriers of these alleles (Cerna *et al*, 1993; Moraes *et al*, 1997). Clinically, FS is characterized by highly superficial, flaccid skin blisters. The thin roofs rapidly rupture, giving rise to painful and

crusted erosions with surrounding erythema. Mucous membrane involvement is rare. Histopathologically, separation in the epidermis can be demonstrated just below the corneal layer. Immunostaining of tissue is characterized by the presence of IgG autoantibodies, predominantly of the IgG4 subtype. The target of these autoantibodies has been shown to be desmoglein 1 (Dsg1), a 160 kDa desmosomal cadherin involved in epidermal cell adhesion (Rock *et al*, 1989; Wheeler *et al*, 1991; Buxton *et al*, 1993). Whereas the role of anti-Dsg1 autoantibodies in FS has been well defined, little is known about the role of T lymphocytes in disease onset and progression. It is thought that T helper cell involvement is required for antibody production by B lymphocytes (Coffinan *et al*, 1988; Stevens *et al*, 1988; Hamano *et al*, 1992). Functionally relevant T cell epitopes within Dsg1 have not yet been conclusively defined, but are thought to be localized to the extracellular domain of the protein (Lin *et al*, 1999).

The fine specificity of T cells for particular major histocompatibility complex-peptide combinations are determined by the physical shape and biochemical properties of the T cell receptor (TCR). Structurally, TCR are heterodimers of α and β , or less commonly γ and δ chains, which fold into peptide loops homologous to immunoglobulin complementarity-determining regions (CDR) encoded by multiple variable (V) regions, joining (J) regions, and in some cases diversity (D) regions. These gene segments are clonally rearranged and joined to a constant (C) exon.

Manuscript received March 1, 2001; revised November 14, 2001; accepted for publication December 3, 2001.

Reprint requests to: Dr. Animesh A. Sinha, Department of Dermatology, Weill Medical College of Cornell University, 510 East 70th Street, LC-702, New York, NY 10021, U.S.A. Email: ans2003@med.cornell.edu

Additional heterogeneity is provided by nucleotide additions at the recombination sites (N region diversity) (Desiderio *et al*, 1984). CDR 1 and 2 are encoded within the V region, whereas the juncture of the V-D-J region (V-J only in α and γ chains) makes up the CDR3, which forms the center of the antigen binding site and plays an essential part in antigen recognition (Chothia *et al*, 1988; Rock *et al*, 1994). The β chain is thought to play the most significant part in defining the antigen specificity of T cells, although the CDR3 region of the α chain does contribute to the structure of the antigen binding and recognition site (Davis and Bjorkman, 1988; Jorgensen *et al*, 1992). A bias in the usage of TCR gene segments may provide insight into the antigenic epitopes responsible for autoimmune initiation and propagation and provide a basis for the development of patient-specific therapies.

PF patient-derived T cell clones used in this study have been previously shown to be Dsg1 specific (Lin *et al*, 1997). To characterize further the role of pathogenic T cells in PF, we have directly characterized the TCR of T cells by polymerase chain reaction (PCR) and nucleotide sequence analysis.

MATERIALS AND METHODS

T cell clones Analysis, derivation, and maintenance of T cell clones has been described previously (Lin *et al*, 2000). Briefly, peripheral blood was collected from FS patients and purified by Ficoll-Hypaque density gradient separation and E-rosetting (Lin *et al*, 1999). T cell response to recombinant Dsg1 was determined by T cell proliferation assay as measured by uptake of [³H]thymidine. Epstein-Barr virus transduced B lymphocyte cell lines, previously developed from FS patients, were used as antigen-presenting cells in the maintenance of cell lines and T cell proliferation assays.

The entire extracellular domain of human Dsg1 was expressed in a baculovirus expression system as previously described (Amagai *et al*, 1995). Briefly, the appropriate complementary DNA (cDNA) sequences were isolated by PCR amplification and cloned into the pVL1393 vector. Recombinant viruses were then generated by homologous recombination according to the manufacturer's instructions (Pharmingen, San Diego, CA). Recombinant Dsg1 (rDsg1) was produced in Sf9 insect cell lines and purified using nickel column chromatography (Ding *et al*, 1999).

T cell proliferation assay T cell responses to rDsg1 or Dsg1-derived peptides were determined by *in vitro* assays based on the uptake of [³H]thymidine as a measurement of proliferation, as previously described (Lin *et al*, 1999). Responses to rDsg1 and peptides was expressed as stimulation index (SI): cpm of cells treated with Dsg1 or peptide per cpm of cells not treated with antigen. An SI greater than or equal to 3 was considered a positive response.

RNA extraction Total RNA was extracted from stimulated T cell clones using TRIzol reagent according to the manufacturer's

specifications (Life Technologies, Rockville, MD). First-strand cDNA was synthesized by reverse transcription using Superscript kit following the manufacturer's protocol (Life Technologies). Complementary DNA was quantitated using ultraviolet spectrometry, and diluted to 20 ng per μ l with dH₂O.

PCR amplification of TCR β chains TCR were amplified by PCR using a 5'-sense primer specific for each of the 24 *TCRBV* families (and two subfamilies) and a conserved 3'-anti-sense *TCRBC* primer as previously described (Panzara *et al*, 1992a, >1992b Longley *et al*, 1995). Five microliters (100 ng) of cDNA was combined with 45 μ l of reaction mix containing 1 \times PCR buffer (Life Technologies), 1.5 mM MgCl₂, 200 μ M deoxyribonucleoside triphosphate, 0.5 μ M of the appropriate *TCRBV*-specific primer, 0.5 μ M of *TCRBC*-specific primer, and 1 unit of Taq DNA polymerase (Life Technologies). Primers were either synthesized by Life Technologies or kindly provided by Dr J. Longley. PCR amplification was carried out using 35 cycles of 93°C for 60 s, 55°C for 60 s, and 72°C for 60 s in a DNA thermocycler (Perkin Elmer, Norwalk, CT). The *TCRBV* gene usage was identified by the presence of a single PCR product from the panel of 26 *TCRBV*-*TCRBC* primer reactions.

Cloning and sequencing of PCR-amplified DNA After PCR amplification and delineation of single *TCRBV* usage, the TCR β chains of each clone were reamplified for 25 cycles using the same conditions described above. PCR products were isolated using a PCR purification kit (Qiagen, Valencia, CA). Fifty nanograms of DNA was adjusted to a volume of 6 μ l, and added to a mixture of 2 μ l of 25 ng per μ l of linearized pCR2.1 vector, 1 μ l T4 DNA ligase, 1 μ l 10 \times ligation buffer. TA cloning was performed according to the manufacturer's specifications (Invitrogen, Carlsbad, CA). The cloned product was then transformed into INV α F cells (Invitrogen). Colonies containing inserts were identified by X-GAL blue-white selection (Life Technologies). Plasmids containing inserts were purified using plasmid Miniprep kits (Qiagen), and analyzed by restriction endonuclease digestion for the presence of proper inserts. Sequencing of cloned inserts was performed at Rockefeller University Protein/DNA Technology Center using 1000 ng of template and 4 pmol of M13 reverse primer (5'-CAG GAA ACA GCT ATG ACC-3'). The experimentally elicited *TCRBC*, *TCRBV*, and *TCRBJ* region sequences were compared to published human TCR sequences (GenBank).

RESULTS

***TCRBV* usage** *TCRBV* gene segments expressed by Dsg1-specific T cell clones were examined by PCR amplification using primers collectively specific for the 26 known human subfamilies (Table I). Collectively, these primers identify the 24 known *TCRBV* families. Additionally, subfamilies of *TCRBV5* (*TCRBV5S1* and *TCRBV5S2*) and *TCRBV13* (*TCRBV13S1* and *TCRBV13S2*) are distinguished by subfamily specific primers.

By PCR analysis, we were able to determine preferences for certain gene segments in 17 T cell clones reactive for the PF

Table I. Sequences of *TCRBV* and *TCRBC* specific primers^a

Family	Specific primer	Family	Specific primer
<i>TCRBV1</i>	5'-GCA CAA CAG TTC CCT GAC TTG CAC-3'	<i>TCRBV13.1</i>	5'-CAA GGA GAA GTC CCC AAT-3'
<i>TCRBV2</i>	5'-TCA TCA ACC ATG CAA GCC TGA CCT-3'	<i>TCRBV13.2</i>	5'-GGT GAG GGT ACA ACT GCC-3'
<i>TCRBV3</i>	5'-GTC TCT AGA GAG AAG AAG GAG CGC-3'	<i>TCRBV14</i>	5'-GTC TCT CGA AAA GAG AAG AGG AAT-3'
<i>TCRBV4</i>	5'-ACA TAT GAG AGT GGA TTT GTC ATT-3'	<i>TCRBV15.1</i>	5'-AGT GTC TCT CGA CAG GCA CAG GCT-3'
<i>TCRBV5.1</i>	5'-ATA CTT GAG TGA GAC ACA GAG AAA C-3'	<i>TCRBV16</i>	5'-AAA GAG TCT AAA CAG GAT GAG TCC-3'
<i>TCRBV5.2-3</i>	5'-TTC CCT AAC TAT AGC TCT GAG CTG-3'	<i>TCRBV17</i>	5'-CAG ATA GTA AAT GAC TTT CAG-3'
<i>TCRBV6.1-3</i>	5'-AGG CCT GAG GGA TCC GTC TC-3'	<i>TCRBV18</i>	5'-GAT GAG TCA GGA ATG CCA AAG GAA-3'
<i>TCRBV7</i>	5'-CCT GAA TGC CCC AAC AGC TCT C-3'	<i>TCRBV19</i>	5'-CAA TGC CCC AAG AAC GCA CCC TGC-3'
<i>TCRBV8</i>	5'-ATT TAC TTT AAC AAC AAC GTT CCG-3'	<i>TCRBV20</i>	5'-AGC TCT GAG GTG CCC CAG AAT CTC-3'
<i>TCRBV9</i>	5'-CCT AAA TCT CCA GAC AAA GCT CAC-3'	<i>TCRBV21</i>	5'-CCT ATT TCT GGC CAT GCT ACC CT-3'
<i>TCRBV10</i>	5'-CTC CAA AAA CTC ATC CTG TAC CTT-3'	<i>TCRBV22</i>	5'-GAT CAG AGA AAA GAG GGA AAC-3'
<i>TCRBV11</i>	5'-TCA ACA GTC TCC AGA ATA AGG ACG-3'	<i>TCRBV23</i>	5'-GAA ATC TCA GAG AAG TCT-3'
<i>TCRBV12</i>	5'-AAA GGA GAA GTC TCA GAT-3'	<i>TCRBV24</i>	5'-TAC CCA GTT TGG AAG C-3'
<i>TCRBC</i>	5'-CTG CTT CTG ATG GCT CAA ACA CAG C-3'		

^aThe primers described here are from several different sources: some of these primers were kindly provided by Dr. J. Longley (Longley *et al*, 1995), whereas others were synthesized according to previously published sequence data (Panzara *et al*, 1992a, >b; Longley *et al*, 1995).

antigen Dsg1 (**Fig 1**). Overall, 10 different *TCRBV* gene segments were used (**Fig 2**). Five of 17 T cell clones from two different patients were found to utilize the same *TCRBV* gene segment—*TCRBV5S1*. No other *TCRBV* subfamily was expressed more than twice in this panel of T cell clones. *TCRBV13.1*, *TCRBV20*, and *TCRBV23* usage was demonstrated in two clones, in each case within clones derived from the same patient. *TCRBV2*, *TCRBV3*, *TCRBV6.1–3*, *TCRBV7*, *TCRBV9*, and *TCRBV14* usage was demonstrated in one clone each. The remaining 16 families and subfamilies were not found to be expressed by any of the T cell clones tested here.

TCRBJ usage To examine TCR gene usage in more detail, and to rule out the possibility that T cell clones from the same patient were derived from the same parental T cells, we sequenced the PCR products used for full-length *TCRBV* analysis. In each case, nucleotide sequencing data confirmed *TCRBV* usage as determined by PCR analysis. In the case of *TCRBV6*, where the primer is collectively specific for the three known subfamilies (*TCRBV6.1*, *TCRBV6.2*, and *TCRBV6.3*), we were able to determine the exact *TCRBV* usage as *TCRBV6.1*.

As the 3'-primer used to amplify the β -chain is specific for the *TCRBC* region, the resulting product also contains the D and J regions located between the V and C regions on the β -chain. *TCRBJ* usage is summarized in **Fig 3** below.

Closer analysis of sequences reveals that clones indeed were not derived from a common parental T cell; we found differential usage of *TCRBJ* gene segments in all cases where identical *TCRBV* segments were utilized (**Table II**). Overall, nine different *TCRBJ* gene segments were found to be expressed in the TCR of FS patient derived T cell clones. *TCRBJ2S5* was found in four of 17, and *TCRBJ1S1* was found in three of 17 T cell clones. Three other gene segments were used twice (*TCRBJ1S6*, *TCRBJ1S2*, and *TCRBJ2S3*) and four were used once (*TCRBJ1S4*, *TCRBJ1S5*, *TCRBJ2S1*, and *TCRBJ2.7*).

Junctional region analysis The junctional region (diversity region and N-diversity region) was identified by comparing experimental *TCRB* sequences to published germline sequences (GenBank) (**Table III**). Sequence analysis demonstrated great diversity in both deduced amino acid sequences and length of the CDR3 junctional region among 17 clones tested. The length of the junctional region ranged from 1 to 8 amino acids in size, and there seemed to be no discernible preference for one size. Amino acid content (deduced from nucleotide sequence) also did not seem to follow a pattern.

Functional analysis T cell response to rDsg1 was determined by proliferation assays. As expected, all 17 rDsg1-specific T cell clones proliferated in response to rDsg1 (see **Table II**), with SI ranging from 3.0 to 17.2. The majority of T cell clones (14 of 17) had an SI

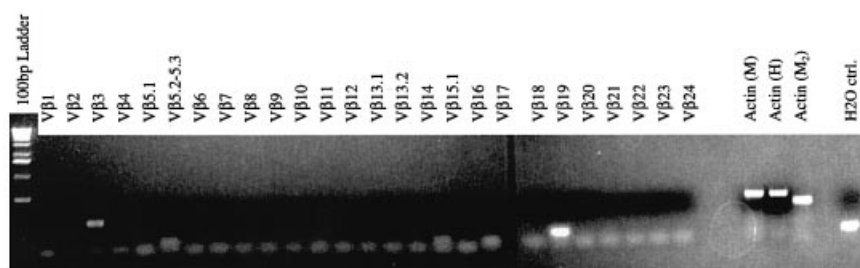
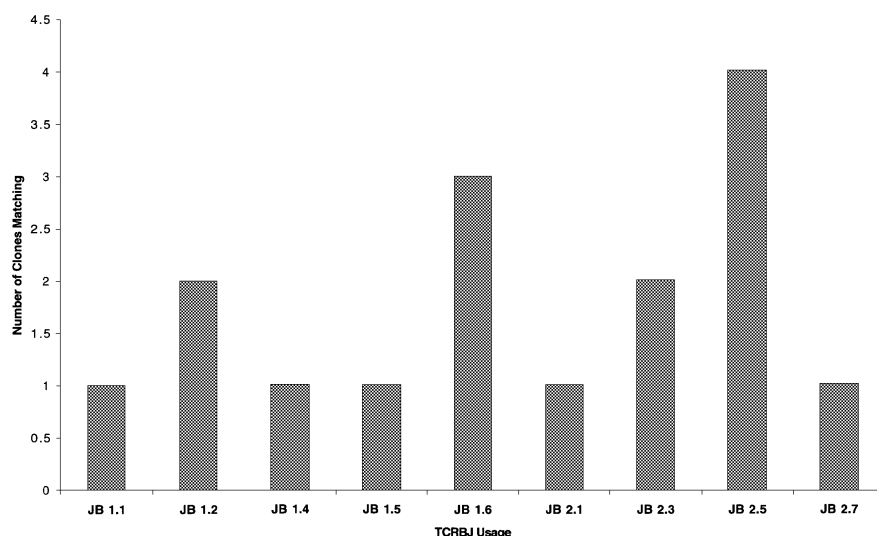


Figure 1. PCR-based screen for *TCRBV* usage. Analysis performed for T cell clone FS12-48 shown here. Each reaction is carried out using 100 ng of cDNA. *TCRBV3* is demonstrated here (lane 4 from left). One kilobase ladder shown in lane 1. Actin (M) and actin (M₂) are PCR controls using separate primers and murine cDNA. Actin (H) is a positive control using actin primers and the TCR cDNA. Note: The bright band seen in lane 19 represents a dimer of primers, rather than a true PCR product. To determine its identity, this band was excised from three separate reactions, and then cloned and sequenced as described above (data not shown). Nucleotide sequencing confirmed that this band represents dimers of the PCR primers used for this reaction. Accordingly, it migrates at a size significantly less than 100 bp, which is distinct from the expected size of the *TCRBV* band (200–250 bp). The only band representing an amplification of a TCR gene is demonstrated in lane 4, and corresponds to *TCRBV3*. In addition to TA cloning mediated sequencing, direct sequencing of the amplified PCR product was performed on one T cell clone (FS12-69) to compare the two methods of sequencing. Sequences of the amplified *TCRB* regions were identical for both methods (data not shown).

Figure 3. *TCRBJ* gene usage. *TCRBJ* gene segment usage in 17 Dsg1-specific PF T cell clones. Gene usage was analyzed by nucleotide sequencing.



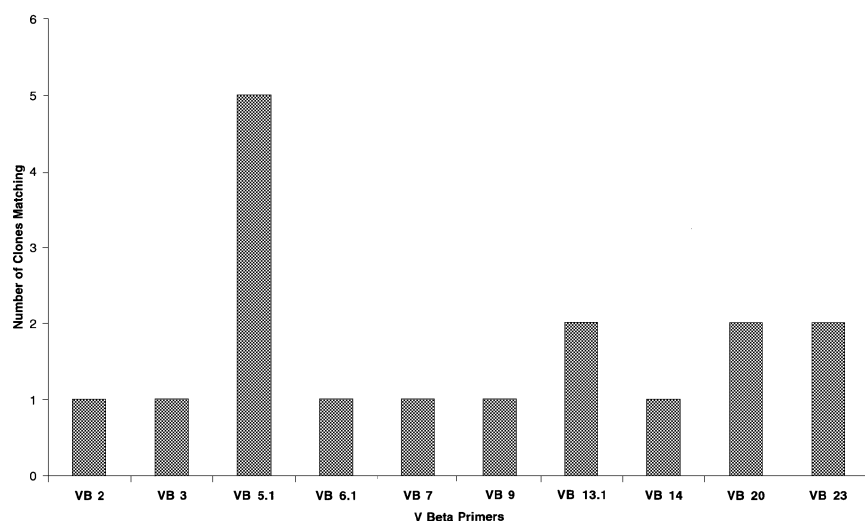


Figure 2. TCRBV gene usage. TCRBV gene segment usage in 17 Dsg1-specific PF T cell clones. Gene usage was analyzed by presence of a unique PCR product and confirmed by DNA sequencing.

Table II. Summary of TCRBV and TCRBJ gene segment usage. HLA typing for patients FS5, FS6, and FS12 was previously described (Lin *et al*, 2000) and is listed for each T cell clone derived from a given patient^a

T cell clone	TCRBV	TCRBJ	Patient	HLA DRB1	HLA DRQ1	Response to rDSG1	Response to p412–426
FS12-24	TCRBV23	TCRBJ2S7	FS12	1402/1602	0301/0301	3.8	
FS12-38	TCRBV2	TCRBJ2S5	FS12	1402/1602	0301/0301	3.1	
FS12-48	TCRBV3	TCRBJ2S1	FS12	1402/1602	0301/0301	3.4	3.8
FS12-58	TCRBV20	TCRBJ1S1	FS12	1402/1602	0301/0301	3.3	
FS12-70	TCRBV5S1	TCRBJ2S5	FS12	1402/1602	0301/0301	3.7	
FS12-75	TCRBV7	TCRBJ1S4	FS12	1402/1602	0301/0301	3.8	
FS12-82	TCRBV23	TCRBJ2S5	FS12	1402/1602	0301/0301	17.2	4.1
FS12-73	TCRBV6S1	TCRBJ1S2	FS12	1402/1602	0301/0301	4.4	3.5
FS12-21	TCRBV14	TCRBJ1S6	FS12	1402/1602	0301/0301	4.1	
FS12-69	TCRBV5S1	TCRBJ1S1	FS12	1402/1602	0301/0301	3.7	
FS12-74	TCRBV5S1	TCRBJ1S6	FS12	1402/1602	0301/0301	3.2	
FS12-84	TCRBV20	TCRBJ2S3	FS12	1402/1602	0301/0301	4.2	
FS5-2	TCRBV5S1	TCRBJ2S5	FS5	1406/1602	0301/0301	3.0	
FS5-74	TCRBV5S1	TCRBJ1S1	FS5	1406/1602	0301/0301	6.9	
FS5-75	TCRBV13S1	TCRBJ1S5	FS5	1406/1602	0301/0301	4.0	
FS5-86	TCRBV13S1	TCRBJ1S2	FS5	1406/1602	0301/0301	5.0	
FS6-81	TCRBV9	TCRBJ2S3	FS6	1406/1602	0301/0301	3.1	

^aThe proliferative response of each clone to rDsg1 has been confirmed and response to peptide Dsg1 412–426 is indicated where applicable (positive response ≥ 3). T cell populations responding to rDsg1 in these three patients were previously characterized (Lin *et al*, 2000). The responding T cells were found to be CD4⁺ and CD45RO⁺, and proliferative responses were HLA-DR restricted. Cytokine profiling indicated that these T cells mount a T helper 2-type response.

ranging from 3.0 to 4.4; however, FS5-86, FS574, and FS12-82 demonstrated higher proliferative responses (SI of 5.0, 6.9, and 17.2, respectively). There seemed to be no correlation between intensity of proliferative response and TCRBV and TCRBJ usage. For example, FS12-69, which uses the same TCRBV–J combination as FS5-74 was found to proliferate with significantly less intensity (SI 3.7 *vs* 6.9).

In an effort to determine the fine specificity of Dsg1 reactive T cell clones, we tested their proliferative response *in vitro* to six synthetic Dsg1-derived peptides, based on epitopes predicted using an major histocompatibility complex binding motif. (Fridkis-Hareli *et al*, 1999) (data not shown). We were able to demonstrate a specific response to one peptide, Dsg1 amino acids 412–426, in three T cell clones (Table II). Further testing of a more comprehensive set of overlapping synthetic peptides for epitope mapping is currently underway.

DISCUSSION

In healthy individuals, the recombination of V (D), J, and C regions of the TCR is thought to be mostly random, although there is

evidence from monozygotic twin studies that background genes and HLA haplotype may influence the recombination preferences of various gene segments (Gulwani-Akolkar *et al*, 1991; Loveridge *et al*, 1991). Germline polymorphisms located within, as well outside, regions coding for TCR genes (Robinson, 1989; Cornelis *et al*, 1993) along with thymic selection (von Boehmer and Kiselow, 1990) have been shown to contribute to the diversity of the peripheral TCR repertoire. Changes within germline TCR sequences may also contribute to autoimmune states in several ways: (i) exon encoded point mutations could create unique specificities relevant to disease; (ii) a larger complement of TCR genes could include sequences predisposing to autoimmune disease; (iii) individuals lacking germline DNA for certain genes could have a TCR repertoire skewed towards autoimmunity; and (iv) alterations in regulatory sequences could result in the overexpression or underexpression of disease relevant TCR.

Not all TCR gene segments are expressed at the same levels in healthy individuals, and gene usage may be skewed within a given CD4⁺ or CD8⁺ T cell population (Liao *et al*, 1989; Davey *et al*, 1991; Akolkar *et al*, 1993). Gene number at the genomic level has not been shown to correlate with TCR expression peripherally

Table III. Sequence alignment and length variability of CDR3 region^a

Clone	<i>TCRBV</i>	Junctional region	<i>TCRBJ</i>
FS12-24	<i>TCRBV23</i> —...CASS	G N R G L G GGAAATCGGGGTTTGGGC	EQYFGPGTRLTVT...— <i>TCRBJ2S7</i>
FS12-38	<i>TCRBV2</i> —...FYIC	S A A V A G E AGTGCGGCGGTAGCGGGGAA	ETQYFGPGTRLLVL...— <i>TCRBJ2S5</i>
FS12-48	<i>TCRBV3</i> —...CASS	S T K R G H TCGACCAAAAGGGGGCAT	EQFFGPGTRLTVL...— <i>TCRBJ2S1</i>
FS12-58	<i>TCRBV20</i> —...CASS	S R N G AGCCGGAATGGC	TEAFFGQGTRLTVV...— <i>TCRBJ1S1</i>
FS12-69	<i>TCRBV5S1</i> —...CASS	A G S W K GCTGGCAGCTGGAAG	EAFFGQGTRLTVV...— <i>TCRBJ1S1</i>
FS12-70	<i>TCRBV5S1</i> —...CASG	V T L S T G GTAACACTCTCCACCGGT	ETQYFGTRLLVL...— <i>TCRBJ2S5</i>
FS12-75	<i>TCRBV7</i> —...CASS	R S A T G R S A CGCAGCGCGACAGGGAGGTCCGA	TNEKLFFGSGTNLSV ...— <i>TCRBJ1S4</i>
FS12-82	<i>TCRBV23</i> —...CAST	T K ACCAAA	ETQYFGTRLLVL...— <i>TCRBJ2S5</i>
FS12-73	<i>TCRBV6S1</i> —...CASS	W T A G Q G S TGGACCGCCGACAGGGGTCT	GYTFGSGTRLTVV...— <i>TCRBJ1S2</i>
FS12-21	<i>TCRBV14</i> —...CASS	V S G T V GTATCCGGGACCGTT	NSPLHFGNGTRLTVT...— <i>TCRBJ1S6</i>
FS12-74	<i>TCRBV5S1</i> —...CASS	F N R D S A A R TTCAACAGGGATTCTGCGGCTCGA	SPLHFGNGTRLTVT...— <i>TCRBJ1S6</i>
FS12-84	<i>TCRBV20</i> —...CAWS	V R N GTAAGAAAT	TQYFGPGTRLTVL...— <i>TCRBJ2S3</i>
FS5-2	<i>TCRBV5S1</i> —...CASS	L CTA	QETQYFGPGTRLLVL...— <i>TCRBJ2S5</i>
FS5-74	<i>TCRBV5S1</i> —...CASS	L G D TTGGGGGAC	TEAFFGQGTRLTVV...— <i>TCRBJ1S1</i>
FS5-75	<i>TCRBV13S1</i> —...CASS	T S R G R G TACTCGCGGGGTAGGGGT	QPQHFGDGTRLSIL...— <i>TCRBJ1S5</i>
FS5-86	<i>TCRBV13S1</i> —...CASS	A A R W GCTGCCAGGTGG	GYTFGSGTRLTVV...— <i>TCRBJ1S2</i>
FS6-81	<i>TCRBV9</i> —...CASS	R L A G Q M CGACTAGCGGGTCAGATG	DTQYFGPGTRLTVL...— <i>TCRBJ2S3</i>

^aAmino acid sequence is deduced from nucleic acid sequence information. The boundaries of *TCRBV* and *TCRBJ* regions were determined by comparison with publicly available germline sequences (GenBank).

(Robinson, 1992). Robinson (1992) found that several variable gene segments (*TCRBV1*, *TCRBV2*, *TCRBV3*, and *TCRBV4*) were expressed at levels more than twice as high as their germline gene number should indicate, and that there was a slight preference for *TCRBJ2* families, particularly *TCRBJ2S1* and *TCRBJ2S7*. Moreover, antigenic history and exposure shape the final expression of the peripheral T cell repertoire as a dynamic process.

Using primers specific for *TCRBV* gene segment families we directly analyzed the *TCRBV* and *TCRBJ* repertoires of T cells derived from PF patients. We were able to identify 10 β -chain V regions and nine β -chain J regions featured at varying frequencies on the TCR of 17 CD4⁺/CD45RO⁺ T helper 2 type T cell clones from three PF patients. Whereas the selection of *TCRBV* and *TCRBJ* gene segments was not randomly distributed, there was not exclusive usage of any single *TCRBV* or *TCRBJ* segment. Analysis of the TCR α chain was not performed for this study, but is being determined in ongoing work. Whereas the α chain may contribute to the shape of the TCR, major histocompatibility complex-peptide specificity appears to be primarily determined by the CDR3 of the β chain (Davis and Bjorkman, 1988; Jorgensen *et al*, 1992).

The preference shown for *TCRBV5S1* (five of 17 clones) and *TCRBJ2S5* (four of 17) may have several implications for the role of the TCR in the initiation of an autoimmune state in PF. It is important to note that, whereas two clones (FS5-2 and FS12-70) from different patients were found to contain the combination *TCRBV5S1*–*TCRBJ2S5*, there did not seem to be an overall preference for this particular *TCRBV*–*J* combination. In fact, three other instances of *TCRBV5S1* usage and two other instances of *TCRBJ2S5* usage were found in conjunction with other *TCRBJ*

and *TCRBV* gene segments, respectively. Duplicate usage of the *TCRBV5S1*–*TCRBJ1S1* combination in two T cell clones from different patients (FS12-69 and FS5-74) represents the only other multiple usage of a particular *TCRBV*–*J* combination. Curiously, for each instance of usage of an identical *TCRBV*–*J* combination, one clone each from patient FS5 and FS12 was included. These two patients each carry a different HLA allele thought to be associated with PF; patient FS5 types as HLA DRB1*1406, whereas patient FS12 carries HLA DRB1*1402 (Cerna *et al*, 1993; Moraes *et al*, 1997). The observation that two patients with different disease susceptibility HLA alleles have autoreactive T cells with identical *TCRBV*–*J* gene usage certainly hints at a preferential usage of certain gene segment combinations in PF. These findings will need to be examined in a larger data set of patients and/or clones to draw significant conclusions.

Close examination of the CDR3 junctional region (see **Table III** for detail) reveals startling heterogeneity. There seems to be no discernible pattern in either length or amino acid preferences in this region. Between 1 and 8 amino acids (deduced from nucleotide sequence) were found to make up this region. Clearly, there does not seem to be a strong selection in favor of a particular amino acid(s).

Overall, *TCRBV* and *TCRBJ* analysis indicates that TCR gene usage in PF shows preferences, but is not completely restricted to a given gene family. Instead, we demonstrate oligoclonality. Moreover, TCR gene usage varies within a given patient, indicating that disease induction and/or progression is not dependent on only one set of TCR gene segments.

A number of investigators have studied TCR gene segment usage in murine models of autoimmune diseases. Nearly mono-

clonal usage of *TCRB8S2* and *TCRB2S5* gene segments was shown in encephalitogen-specific T cells in the (PL/J) mouse model of experimental autoimmune encephalomyelitis (Acha-Orbea *et al*, 1988; Zamvil *et al*, 1988). Similarly, *TCRBV8S1* and *TCRBV8S2* were also found in 60% of T cells isolated from enlarged lymph nodes of MRL-lpr/lpr lupus mice (Singer *et al*, 1986). *TCRBV8S2*, *TCRBV10*, and *TCRBV12* were found to be oligoclonally expressed throughout disease initiation and progression of experimental autoimmune myocarditis (Matsumoto *et al*, 2000).

Analysis of human diseases has shown greater diversity, frequently showing oligoclonal selection, but rarely monoclonal gene usage. It has been shown that, whereas there seems to be a preference for *TCRBV8S2* in multiple sclerosis lesions, there is multiple *TCRBV* and *TCRBJ* gene segment usage seen overall (Oksenberg *et al*, 1993; Lodge *et al*, 1994). An early study of TCR gene diversity of peripheral blood T cells and lesional skin in psoriasis showed no skewing of the repertoire towards a particular set of V-region genes (Moss *et al*, 1997); however, Vekony *et al* (1997) demonstrated a slight preference for *TCRBV2* and *TCRBV6* in early psoriatic lesions, whereas Chang *et al* (1994) reported increased usage of *TCRBV3S1* and *TCRBV13S1* in CD8⁺ cells found in active lesions. Similar evidence of some oligoclonal preference has been reported in systemic lupus erythematosus (Holbrook *et al*, 1996), alopecia areata (*TCRBV5S2*, *TCRBV14*, *TCRBV22*) (Szafer *et al*, 1995), and rheumatoid arthritis (*TCRBV12*, *TCRBV3*, *TCRBV14*, *TCRBV17*) (Howell *et al*, 1991; DerSimonian *et al*, 1993; Hall *et al*, 1998). Functional relevance remains to be established in each case. Similarly, it is difficult at this point to assess whether our demonstration of oligoclonal *TCRBV* and *TCRBJ* preference in PF T cell clones is relevant for disease induction or reflects T cell specificities generated as a result of epitope spreading at a point further along in disease progression.

In an effort to determine whether there is a correlation between epitope-specific response and *TCRBV* gene usage, we tested patient-derived T cell clones for proliferative responses to several Dsg1-derived synthetic peptides. We identified a 15 amino acid peptide (Dsg1 amino acids 412–426), which elicited a response in three T cell clones tested (FS12-48, FS12-82, and FS12-73). Interestingly, none of these three T cell clones show duplicate usage of either *TCRBV* or *TCRBJ* gene segments. There also seemed to be no discernible pattern in the amino acid content of the junctional region. These findings are relevant for the prospect of *TCRBV* (or other gene segment) specific immunotherapy. The possibility of multiple TCR subspecificities capable of recognizing even a single relevant epitope suggests that it will be difficult to easily target the complement of autoreactive T cells in a given disease.

Still, recent advances in immunotherapy highlight the therapeutic prospects based on precisely identifying the TCR involved in autoimmune disease. Treatment with a peptide specific for the *TCRBV* region (Acha-Orbea *et al*, 1988; Urban *et al*, 1988), or vaccination with synthetic peptides based on particular *TCRBV* region sequences (Howell *et al*, 1989; Vandenbark *et al*, 1989; Offner *et al*, 1991) have been shown to be effective in downregulating the immune response in experimental autoimmune encephalomyelitis. More recently, clinical human trials in rheumatoid arthritis using peptides based on *TCRBV3*, *TCRBV14*, and *TCRBV17* have shown that such vaccinations may be effective in the prevention of human disease. Similarly, trials using a *TCRBV6* CDR3 region peptide vaccine in patients with multiple sclerosis have also shown promising results (Gold *et al*, 1997). Vaccination with DNA coding for disease-related *TCRBV* gene segments have also shown promise in experimental autoimmune encephalomyelitis (*TCRBV8.2*) (Waisman *et al*, 1996) and experimental autoimmune myocarditis (*TCRBV8.2* and *TCRBV10*) (Matsumoto *et al*, 2000).

A major goal of characterizing T cell specificities in autoimmunity is to understand the molecular events that lead to

autoimmune disease initiation and propagation. A critical link in this chain of events is the specificity of the TCR for a given antigenic epitope. Whereas the analysis of T cell clone TCR usage may provide important insights into the events that govern the molecular interactions of the trimolecular complex, it is limited by the fact that it can only reflect a narrow snapshot of a patient's entire T cell complement and provides only indirect evidence of antigen specificity. The difficulty of deriving and maintaining T cell clones effectively renders this approach impractical for screening larger patient populations or a comprehensive sample of T cells. TCR spectratyping aims to identify gene usage on a broader scale in peripheral blood and tissue, yet fails to provide information on antigen-specific interactions. Recent advances in T cell analysis are aimed to improve the sensitivity and accuracy of determining T cell activation and specificity. Flow cytometric assessment of intracellular cytokine release can be used to detect antigen-specific T cells following stimulation with protein antigens (Waldrop *et al*, 1997) or even peptides (Kern *et al*, 1998). Fluorescently labeled tetrameric major histocompatibility complex–peptide complexes are increasingly being used to determine directly and more accurately the number of peripheral antigen-specific T cells in HLA class I and class II systems (Altman *et al*, 1996; Novak *et al*, 1999; Kwok *et al*, 2000). To advance our understanding of the role of T cells in autoimmune disease, future work will need to incorporate sequence and structural detail with large-scale analysis of the TCR fine specificity and functional activity.

We would like to thank Dr. J. Longley for kindly providing some of the primers used in this experiment and technical assistance.

REFERENCES

- Acha-Orbea H, Mitchell DJ, Timmermann L, *et al*: Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell* 54:263–273, 1988
- Akolkar PN, Gulwani-Akolkar B, Pergolizzi R, Bigler RD, Silver J: Influence of HLA genes on T cell receptor V segment frequencies and expression levels in peripheral blood lymphocytes. *J Immunol* 150:2761–2773, 1993
- Altman JD, Moss PAH, Goulder PJR, *et al*: Phenotypic analysis of antigen-specific T lymphocytes. *Science* 274:94–96, 1996
- Amagai M, Hashimoto T, Green KJ, Shimizu N, Nishikawa T: Antigen-specific immunoadsorption of pathogenic autoantibodies in pemphigus foliaceus. *J Invest Dermatol* 104:895–901, 1995
- Bastuji-Garin S, Souissi R, Blum L, *et al*: Comparative epidemiology of pemphigus in Tunisia and France: unusual incidence of pemphigus foliaceus in young Tunisian women. *J Invest Dermatol* 104:302–305, 1995
- von Boehmer H, Kieselow P: Self-nonself discrimination by T cells. *Science* 248:1369–1373, 1990
- Buxton RS, Cowin P, Franke WW, *et al*: Nomenclature of the desmosomal adherens. *J Cell Biol* 121:481–483, 1993
- Cerna M, Fernandez-Vina M, Friedman H, Moraes JR, Moraes M, Diaz L, Stastny P: Genetic markers for susceptibility to endemic Brazilian pemphigus foliaceus (Fogo Selvagem) in Xavante Indians. *Tissue Antigens* 42:138–140, 1993
- Chang JC, Smith LR, Froning KJ, *et al*: CD8⁺ T cells in psoriatic lesions preferentially use T-cell receptor V beta 3 and/or V beta 13.1 genes. *Proc Natl Acad Sci USA* 91:9282–9286, 1994
- Chothia C, Boswell DR, Lesk AM: The outline structure of the T-cell alpha beta receptor. *EMBO J* 7:3745–3755, 1988
- Coffman RL, Seymour BW, Leberman DA, *et al*: The role of helper T cell products in mouse B cell differentiation and isotype regulation. *Immunol Rev* 102:5–28, 1988
- Cornelis F, Pile K, Loveridge J, Moss P, Harding R, Julier C, Bell J: Systematic study of human alpha beta T cell receptor V segments shows allelic variations resulting in a large number of distinct T cell receptor haplotypes. *Eur J Immunol* 23:1277–1283, 1993
- Davey MP, Meyer MM, Munkirs DD, Babcock D, Braun MP, Hayden JP, Bakke AC: T-cell receptor variable beta genes show differential expression in CD4 and CD8 T cells. *Hum Immunol* 32:194–202, 1991
- Davis MM, Bjorkman PJ: T-cell antigen receptor genes and T-cell recognition. *Nature* 334:395–402, 1988
- DerSimonian H, Sugita M, Glass DN, Maier AL, Weinblatt ME, Reme T, Brenner MB: Clonal V alpha 12.1+ T cell expansions in the peripheral blood of rheumatoid arthritis patients. *J Exp Med* 177:1623–1631, 1993
- Desiderio SV, Yancopoulos GD, Paskind M, *et al*: Insertion of N regions into heavy-chain genes is correlated with expression of terminal deoxynucleotidyl transferase in B cells. *Nature* 311:752–755, 1984
- Diaz LA, Sampaio SA, Rivitti EA, *et al*: Endemic pemphigus foliaceus (fogo

- selvagem). II. Current and historic epidemiologic studies. *J Invest Dermatol* 92:4–12, 1989
- Ding X, Diaz LA, Fairley JA, Giudice GJ, Liu Z: The anti-desmoglein 1 autoantibodies in pemphigus vulgaris sera are pathogenic. *J Invest Dermatol* 112:739–743, 1999
- Fridkis-Hareli M, Neveu JM, Robinson RA, et al: Binding motifs of copolymer 1 to multiple sclerosis- and rheumatoid arthritis-associated HLA-DR molecules. *J Immunol* 162:4697–4704, 1999
- Gold DP, Smith RA, Golding AB, et al: Results of a phase I clinical trial of a T-cell receptor vaccine in patients with multiple sclerosis. II. Comparative analysis of TCR utilization in CSF T-cell populations before and after vaccination with a TCRV beta 6 CDR2 peptide. *J Neuroimmunol* 76:29–38, 1997
- Gulwani-Akolkar B, Posnett DN, Janson CH, et al: T cell receptor V-segment frequencies in peripheral blood T cells correlate with human leukocyte antigen type. *J Exp Med* 174:1139–1146, 1991
- Hall FC, Thomson K, Procter J, McMichael AJ, Wordsworth BP: TCR beta spectratyping in RA: evidence of clonal expansions in peripheral blood lymphocytes. *Ann Rheum Dis* 57:319–322, 1998
- Hamano T, Asano Y, Iwasaki T, Yamasaki T, Hase K, Kakishita E: Direct interaction between an antigen-specific B cell clone and an MHC class II-reactive helper T cell clone. *J Leuk Biol* 52:89–96, 1992
- Holbrook MR, Tighe PJ, Powell RJ: Restrictions of T cell receptor beta chain repertoire in the peripheral blood of patients with systemic lupus erythematosus. *Ann Rheum Dis* 55:627–631, 1996
- Howell MD, Winters ST, Olee T, Powell HC, Carlo DJ, Brostoff SW: Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides. *Science* 246:668–670, 1989
- Howell MD, Diveley JP, Lundeen KA, Esty A, Winters ST, Carlo DJ, Brostoff SW: Limited T-cell receptor beta-chain heterogeneity among interleukin 2 receptor-positive synovial T cells suggests a role for superantigen in rheumatoid arthritis. *Proc Natl Acad Sci USA* 88:10921–10925, 1991
- Jorgensen JL, Esser U, Fazekas de St Groth B, Reay PA, Davis MM: Mapping T-cell receptor-peptide contacts by variant peptide immunization of single-chain transgenics. *Nature* 355:224–230, 1992
- Kern F, Surel IP, Brock C, et al: T-cell epitope mapping by flow cytometry. *Nat Med* 4:975–978, 1998
- Kwok WW, Liu AW, Novak EJ, et al: HLA-DQ tetramers identify epitope-specific T cells in peripheral blood of herpes simplex virus type 2-infected individuals: direct detection of immunodominant antigen-responsive cells. *J Immunol* 164:4244–4249, 2000
- Liao NS, Maltzman J, Raulet DH: Positive selection determines T cell receptor V beta 14 gene usage by CD8+ T cells. *J Exp Med* 170:135–143, 1989
- Lin MS, Swartz SJ, Lopez A, et al: Development and characterization of desmoglein-3 specific T cells from patients with pemphigus vulgaris. *J Clin Invest* 99:31–40, 1997
- Lin MS, Gharia MA, Swartz SJ, Diaz LA, Giudice GJ: Identification and characterization of epitopes recognized by T lymphocytes and autoantibodies from patients with herpes gestationis. *J Immunol* 162:4991–4997, 1999
- Lin MS, Fu CL, Aoki V, et al: Desmoglein-1-specific T lymphocytes from patients with endemic pemphigus foliaceus (fogo selvagem). *J Clin Invest* 105:207–213, 2000
- Lodge PA, Allegretta M, Steinman L, Sriram S: Myelin basic protein peptide specificity and T-cell receptor gene usage of HPRT mutant T-cell clones in patients with multiple sclerosis. *Ann Neurol* 36:734–740, 1994
- Longley JL, Tyrrell SZ, Lu J, et al: Malignant and normal T cells show random use of T-cell receptor alpha chain variable regions in patients with cutaneous T-cell lymphoma. *J Invest Dermatol* 105:62–64, 1995
- Loveridge JA, Rosenberg WM, Kirkwood TB, Bell JI: The genetic contribution to human T-cell receptor repertoire. *Immunology* 74:246–250, 1991
- Matsumoto Y, Jee Y, Sugisaki M: Successful TCR-based immunotherapy for autoimmune myocarditis with DNA vaccines after rapid identification of pathogenic TCR. *J Immunol* 164:2248–2254, 2000
- Moraes ME, Fernandez-Vina M, Lazaro A, et al: An epitope in the third hypervariable region of the DRB1 gene is involved in the susceptibility to endemic pemphigus foliaceus (fogo selvagem) in three different Brazilian populations. *Tissue Antigens* 49:35–40, 1997
- Morini JP, Jomaa B, Gorgi Y, Saguem MH, Nouira R, Roujeau JC, Revuz J: Pemphigus foliaceus in young women. An endemic focus in the Sousse area of Tunisia. *Arch Dermatol* 129:69–73, 1993
- Moss P, Charnley P, Mulvihill E, et al: The repertoire of T cell antigen receptor beta-chain variable regions associated with psoriasis vulgaris. *J Invest Dermatol* 109:14–19, 1997
- Novak EJ, Liu AW, Nepom GT, Kwok WW: MHC class II tetramers identify peptide-specific human CD4(+) T cells proliferating in response to influenza A antigen. *J Clin Invest* 104:R63–R67, 1999
- Offner H, Hashim GA, Vandenbark AA: T cell receptor peptide therapy triggers autoregulation of experimental encephalomyelitis. *Science* 251:430–432, 1991
- Oksenberg JR, Panzara MA, Begovich AB, et al: Selection for T-cell receptor V beta-D beta-J beta gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. *Nature* 362:68–70, 1993
- Panzara MA, Gussoni E, Steinman L, Oksenberg JR: Analysis of the T cell repertoire using the PCR and specific oligonucleotide primers. *Biotechniques* 12:728–735, 1992a
- Panzara MA, Oksenberg JR, Steinman L: The polymerase chain reaction for detection of T-cell antigen receptor expression. *Curr Opin Immunol* 4:205–210, 1992b
- Robinson MA: Allelic sequence variations in the hypervariable region of a T-cell receptor beta chain: correlation with restriction fragment length polymorphism in human families and populations. *Proc Natl Acad Sci USA* 86:9422–9426, 1989
- Robinson MA: Usage of human T-cell receptor V beta, J beta, C beta, and V alpha gene segments is not proportional to gene number. *Hum Immunol* 35:60–67, 1992
- Robledo MA, Prada S, Jaramillo D, Leon W: South American pemphigus foliaceus. study of an epidemic in El Bagre and Nechi, Colombia 1982–86. *Br J Dermatol* 118:737–744, 1988
- Rock B, Martins CR, Theofilopoulos AN, et al: The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem). *N Engl J Med* 320:1463–1469, 1989
- Rock EP, Sibbald PR, Davis MM, Chien YH: CDR3 length in antigen-specific immune receptors. *J Exp Med* 179:323–328, 1994
- Singer PA, McEvilly RJ, Noonan DJ, Dixon FJ, Theofilopoulos AN: Clonal diversity and T-cell receptor beta-chain variable gene expression in enlarged lymph nodes of MRL-lpr/lpr lupus mice. *Proc Natl Acad Sci USA* 83:7018–7022, 1986
- Squiquera HL, Diaz LA, Sampaio SA, et al: Serologic abnormalities in patients with endemic pemphigus foliaceus (Fogo selvagem), their relatives, and normal donors from endemic and non-endemic areas of Brazil. *J Invest Dermatol* 91:189–191, 1988
- Stevens TL, Bossie A, Sanders VM, Fernandez-Botran R, Coffman RL, Mosmann TR, Vitetta ES: Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature* 334:255–258, 1988
- Szafer F, Price VH, Oksenberg JR, Steinman L: T-cell receptor repertoire V beta in alopecia areata. *J Invest Dermatol* 104:22S–24S, 1995
- Urban JL, Kumar V, Kono VH, et al: Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell* 54:577–592, 1988
- Vandenbark AA, Hashim G, Offner H: Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. *Nature* 341:541–544, 1989
- Vekony MA, Holder JE, Lee AJ, Horrocks C, Eperon IC, Camp RD: Selective amplification of T-cell receptor variable region species is demonstrable but not essential in early lesions of psoriasis vulgaris: analysis by anchored polymerase chain reaction and hypervariable region size spectratyping. *J Invest Dermatol* 109:5–13, 1997
- Waisman A, Ruiz PJ, Hirschberg DL, et al: Suppressive vaccination with DNA encoding a variable region gene of the T-cell receptor prevents autoimmune encephalomyelitis and activates Th2 immunity. *Nat Med* 2:899–905, 1996
- Waldrop SL, Pitcher CJ, Peterson DM, Maino VC, Picker LJ: Determination of antigen-specific memory/effector CD4+ T cell frequencies by flow cytometry: evidence for a novel, antigen-specific homeostatic mechanism in HIV-associated immunodeficiency. *J Clin Invest* 99:1739–1750, 1997
- Wheeler GN, Parker AE, Thomas CL, et al: Desmosomal glycoprotein DGI, a component of intercellular desmosome junctions, is related to the cadherin family of cell adhesion molecules. *Proc Natl Acad Sci USA* 88:4796–4800, 1991
- Zamvil SS, Mitchell DJ, Lee NE, et al: Predominant expression of a T cell receptor V beta gene subfamily in autoimmune encephalomyelitis. *J Exp Med* 167:1586–1596, 1988